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G Protein Heterodimers: New Structures Propel New Questions

Minireview

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The heterotrimeric G proteins transmit signals from a variety of cell surface receptors to enzymes and channels (reviewed by Neer, 1995). The heterotrimer consists of an α subunit that binds and hydrolyzes GTP and a pair of proteins, β and γ, that are tightly associated with each other. The GTP-activated Gα subunits dissociate from Gβγ, and both subunits then activate their respective effectors. The subunits stay separated until GTP is hydrolyzed to GDP, whereupon they reassemble and both become inactive. Therefore, the contact surface between Gα and Gβγ has major regulatory importance.

Two groups have now independently determined the structure of Gαβγ and free Gβγ (Wall et al., 1995 [$G\alpha_{i1}\beta_1\gamma_2$]; Lambright et al., 1996 [$G\alpha_t$ chimera $\beta_1\gamma_1$]; Sondek et al., 1996 [free $G\beta_1\gamma_1$]). The new structures (Figure 1) reveal that the conformation of the GDP-liganded α subunit in the heterotrimer is different from the GDP-liganded α subunit alone (and also different from the crystallized GTPγS-liganded form). They also reveal that the Gβ subunit folds into a highly symmetric β propeller. Each propeller blade consists of a small four-stranded twisted β sheet, the innermost β strand being nearly parallel to the axis of the central tunnel (see schematic in Figure 2). This central channel or tunnel is lined with the unsatisfied hydrogen donors and acceptors from the edge of the inner (a) strand of each propeller blade; possibly these are water solvated. A number of unrelated proteins form similar circular structures with a rather flat top and bottom, a central tunnel, and four or more blades (Faber et al., 1995). The remarkable similarity among β propeller structures is illustrated in Figure 3, which shows equivalent views of Gβ and methylamine dehydrogenase, a bacterial protein with no obvious sequence or functional relationship to Gβ (Chen et al., 1995). The Gβ subunit contains seven repetitions of a highly conserved sequence corresponding to each of the propeller blades. The repeating sequence (diagrammed in Figure 4) is characterized by a conserved core of amino acids usually bounded by Gly–His (GH) and Trp–Asp (WD) and separated by a variable length region. The repeating unit occurs four to ten times in a wide variety of proteins (about 50 kinds) with apparently unrelated cellular functions (Neer et al., 1994). Given the conserved amino acid sequence among all these proteins, it is likely that all form the same propeller fold.

The αβ Contact Surface

The docking of Gα to Gβγ involves extensive contacts: binding of the Gα N-terminal α helix to the side of the Gβ propeller parallel to its central tunnel and binding of the catalytic domain of Gα to the top surface of the β propeller (see Figure 1). Removal of the N-terminal α helix from Gα prevents formation of Gαβγ heterotrimers (reviewed by Neer, 1995). The catalytic domain binds to Gβ through a region called switch II, previously known to change conformation upon nucleotide binding and to be chemically cross-linkable to the Gβγ subunit (Garcia-Higuera et al., 1996, and references therein). A number of salt bridges and the fit of Gβ Trp-99 into a hydrophobic pocket on Gα determine binding of Gα to the top of the propeller. Mutation of an equivalent Trp in yeast Gβ disrupts Gβγ binding to Gα, leading to constitutive activation of the yeast mating pathway (see Wall et al., 1995; Lambright et al., 1996).

GDP-liganded Gα in a Gαβγ heterotrimer is different from GDP-liganded Gα alone (see references in Wall et al., 1995; Lambright et al., 1996). This may be partly due to differences in crystal structures and crystal–crystal

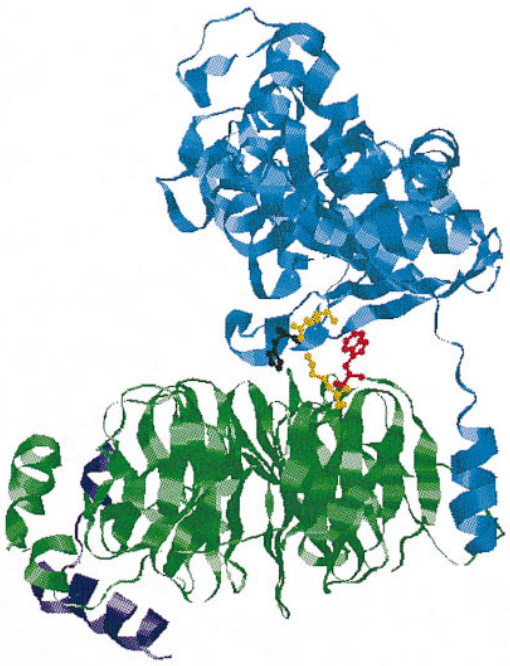

Figure 1. A Ribbon Diagram of the Gαβγ

We thank Dr. S. Sprang for providing the coordinates. The view is directly perpendicular to the Gβ propeller's central symmetry axis. The Gα subunit is displayed in light blue, the Gβ in green, and the Gγ in dark blue. Four Gα–Gβγ interactions are shown: the packing of the Gα N-terminal helix onto the side of the β propeller; the protrusion of the Gβ Trp-99 (in red) into the Gα lower surface; one of the Gα–Gβ salt bridges (in yellow); and, finally, the placement of the Gα switch II His-213 directly above the propeller's central tunnel (in black). We have called the narrower face of the propeller the top, as in Lambright et al. (1996) and Sondek et al. (1996); Wall et al. (1995) use the opposite designation.

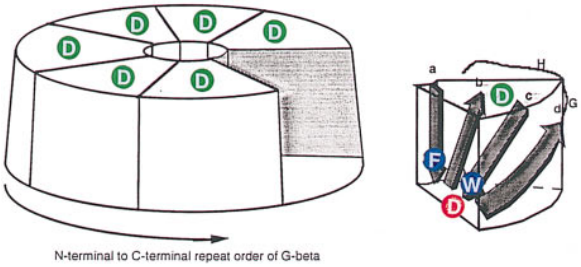

Figure 2. A Schematic of the Gβ Propeller Structure

The schematic shows the relative placement of the four sequential β strands in each of the seven blades. Also shown are the key WD repeat amino acids (see Figure 4). The seven symmetrically placed surface Asps in the tight two to three residue turn between strands b and c are indicated by green cirles on the top surface of Gβ. These are not the D of WD. The highly conserved aromatics at the lower ends of strands a and c are shown by blue circles. The Asp of the defining WD, potentially exposed on the propeller's wider bottom surface, is indicated by a red circle.

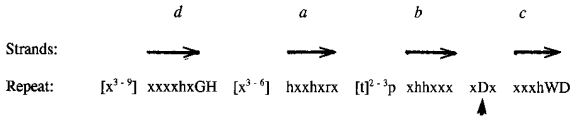

Figure 4. The WD Repeat of Gβ subunits

The strands that make up a propeller blade are termed a, b, c, and d according to Wall et al. (1995) (Lambright et al. [1996] and Sondek et al. [1996] term them 1, 2, 3, and 4) and are indicated by the long arrows. The residues in the repeat are marked: x, a nonconserved position; h, a conserved hydrophobic position; r, a conserved aromatic; p, a conserved polar position; t, a tight turn containing Gly, Pro, Asp, or Asn. The superscripts indicate the range of residues observed in the various known Gβ subunits. The arrowhead identifies the highly conserved Asp.

contacts, as discussed by Bourne (1995). In the GTP-bound form, the small α2 helix (switch II) at the interface of Gα and Gβγ is stabilized by interactions with the γ phosphate of GTP. Once that phosphate is cleaved and, presumably, released, the switch II helix rotates, exposing hydrophobic residues and forming the pocket for the Gβ Trp-99.

In the heterotrimer, the ring of His-213 in $G\alpha_{i1}$ (or His-209 in $G\alpha_t$) in switch II is positioned directly above the Gβ propeller's central tunnel. This position suggests an interaction of the switch II region with the central tunnel. Such an interaction predicts a strong pH dependence for Gα subunit activation, perhaps even over the intracellular pH range. Other propeller proteins use the tunnel entrance to coordinate a ligand and so helps a second catalytic unit or domain carry out its function. The ligand can be a calcium ion (in collagenase), a heme (in hemopexin), a quinone (in methanol dehydrogenase), or a cofactor bound to a separate chain (the tryptophan–tryptophylquinone bound to the L chain of methylamine dehydrogenase). The Gβγ subunit can be considered to "coordinate" the Gα His-213, an ionizable group in switch II, perhaps to hold Gα in the inactive conformation.

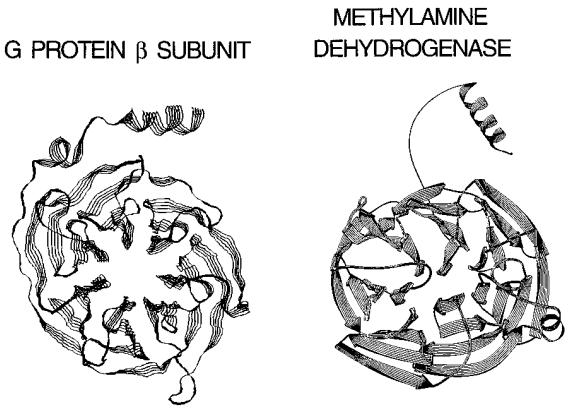

Figure 3. Diagrams of Methylamine Dehydrogenase and Gβ

Methylamine Dehydrogenase is shown on the right (adapted from Chen et al., 1992), and Gβ is shown on the left (from coordinates of Wall et al., 1995). The Gγ subunit is not shown.

The βγ Subunit

As in so many other cases, a newly determined structure provides answers to many old puzzles and creates new ones. For example, the β subunit is cut only once by trypsin, even though it contains many potential cleavage sites. We now know that a single large loop exposes this cleavage site. Next, the tight binding of Gγ to Gβ can be explained by the finding that Gγ makes very few contacts with itself. Instead, it is stretched along the side and bottom of the Gβ subunit contacting blades 5, 6, 7, and 1. $G\gamma_1$ seems to be better ordered than $G\gamma_2$ since more of its residues are visible (Wall et al., 1995; Sondek et al., 1996). A Gγ-like subunit is not unique to propeller proteins with WD repeats. A small protein is also extended across the wide end of methanol dehydrogenase, a seven-bladed propeller. Like Gγ, it can only be separated from the propeller by denaturation, but its function is not known (Xia et al., 1992).

In the structure of Lambright et al. (1996), the N-terminus of $G\alpha_t$ (that is acylated) and the C-terminus of Gγ (that is prenylated) are 18 Å apart on a common face of the Gαβγ heterotrimer. Lambright et al. (1996) and Wall et al. (1995) propose that the hydrophobic modifications insert simultaneously into the lipid bilayer to stabilize the heterotrimer rather than binding to hydrophobic sites on the G proteins.

Among the many new puzzles is understanding what holds a propeller together and how it forms. Both Wall et al. (1995) and Lambright et al. (1996) point out that the very highly conserved Asp between strands b and c in the WD repeats (not the Asp in WD; see Figures 2 and 4) are involved in the formation of an inter- and intrablade hydrogen bond triad, involving His in the GH motif and a Ser/Thr in the blade b strands. Such a triad appears to occur at four out of seven blade interfaces in Gβ. While this Asp is the most highly conserved WD repeat residue (occurring in 86% of 296 repeats we analyzed), the His is only found in about 60% of repeats and the Thr/Ser in even fewer. The propeller fold seems quite forgiving, since propellers can form without any of these amino acids. Therefore, the almost total conservation of the Asp indicates it may have additional roles. Two other highly conserved positions in WD repeats, the central aromatic found near the end of the first (or a) strand of each blade and the defining C-terminal Trp,

have essential structural roles. Both these aromatics are positioned such that their rings lie nearly parallel to the propeller's central axis, forming the rather flat hydrophobic lower face of each propeller blade. This hydrophobic surface provides much of the required structural stability between neighboring propeller blades, but is not the only stabilizing force.

All of the propeller proteins have evolved ways to snap or "velcro" the circle closed. In Gβ, the velcro is provided by the outermost (d) strand in the last blade. This d strand comes from the sequence just prior to the inner (a) strand of the first blade and closes the ring. While some unrelated propeller proteins use this same closure, others have developed variants, including forming a disulfide bond between the first and last blades of small four-bladed proteins (hemopexin and collagenase) (Xia et al., 1992; Li et al., 1995). The apparent necessity for a method of closure suggests either that the circular structures are inherently unstable or that they must be velcroed shut to guard against the domain swapping that might cause assembly in the wrong order (Bennett et al., 1994). These fascinating structures may require special pathways or special folding dynamics to assemble the blades correctly.

Specialization of WD Repeats in Gβ

Analysis of the repeating sequences of Gβ subunits taken from organisms widely separated by evolutionary time suggested that the different repeating units in Gβ became specialized very early and that this specialization has been highly conserved over at least the last 1.2 billion years (Neer et al., 1994). The specializations occurred in the conserved core of the repeating units, as well as in the regions between the conserved cores. The structure of Gβ now allows us to associate these repeat specializations with particular structural positions and possible function.

The α subunit is located asymmetrically over the tunnel in Gβ (see Figure 1). The character of the surfaces of blades 1 and 2 (which include Trp-99; discussed above) have been conserved to preserve the binding to Gα. The outer strands of blades 1 and 7 must retain the ability to bind the N-terminal α helix of Gα, while the large loop extending from blade 2 may help to orient that helix along the side of Gβ. The Gγ subunit extends along the wide surface of Gβ, making contact with blades 5, 6, 7, and 1. The loops and turns on this surface must retain the ability to bind Gγ and to discriminate among different Gγs.

Now that we know how the Gα, Gβ, and Gγ subunits go together, a major question is where do receptors, effectors, and other proteins bind to Gβγ. Some repeat specializations probably adapt the basic structure for interaction with other proteins. For example, there is an insert of three amino acids into repeat 6 that helps generate a very hydrophobic patch that runs from near the top center of the propeller down the side of blades 5 and 6. Such a hydrophobic patch seems made for binding a partner protein.

Unlike Gα, Gβγ does not undergo any conformational changes between the heterotrimeric and the dimeric state (Sondek et al., 1996), although it is possible that binding to an effector may induce a conformational change. Since Gα blocks interaction of Gβγ with all its known effectors, without inducing a conformational change in Gβγ, it is likely that Gα sterically interferes with binding of many effectors. Conversely, a large fragment of one Gβγ-regulated effector, the β-adrenergic receptor kinase, interferes not only with Gβγ activation of several other effectors but also with binding of Gα to Gβγ (Inglese et al., 1994). While Gα- and effector-binding sites may overlap, they are surely not identical. For example, a small peptide derived from one Gβγ effector, adenylyl cyclase, interferes with activation of other effectors, but not with Gα binding (Chen et al., 1995). Perhaps, it is too small to reach from its binding site to that of Gα. Sondek et al. (1996) propose that the N-terminal coiled coil forms part of the effector site, since mutations in this region of yeast Gβγ have a dominant negative phenotype in cells with wild-type βγ. However, the coiled coil is far from the Gα-binding site, so it is unclear how Gα would block an effector that binds there. In contrast with effectors, receptors bind to the Gαβγ heterotrimer so they cannot bind to regions of Gβ now known to be occupied by Gα and Gγ. Likely candidates are blades 6 and 7 as discussed by Wall et al. (1995).

Generalizations

Clearly, considerable insight has been and will be provided into the workings of the large G protein family by these new structural determinations, yet their implications and probable utility go much further. There are a large number of proteins that contain four to ten clearly recognizable WD repeats, but that are functionally unrelated to the G proteins. There is good reason to think that they all will fold into propellers, sharing much with the new Gβ structures. Thus, Gβ will provide a powerful model for experimental design by suggesting various structural hypotheses to explain the wide range of functions represented by this extended family. The Gβ structure now allows us to distinguish those parts of other WD repeat proteins that are important for maintaining the propeller structure itself from those parts that probably make up surface loops and turns. Within a functionally related family of WD repeat proteins, surface residues that are conserved over long evolutionary periods point to regions important for function and identify useful targets for mutagenesis.

The Gβ structure provides an apparently rigid scaffold for various surface embellishments. Absence of major conformational change in the propeller structure itself seems to be characteristic of the group. For example, another propeller protein, galactose oxidase, that has been crystallized under various conditions does not show major conformational changes (Ito et al., 1994). None of the approximately 50 WD repeat proteins are known to be enzymes, but most of the non–WD repeat propeller proteins do form part of enzymes. In most cases, the propellers do not make up the entire catalytic domain. One exception is influenza neuraminidase (see references in Faber et al., 1995), where the residues making up the active site are part of the propeller. Probably, the necessary protein motions are contained in the large surface loops, not in the motion of the propeller blades with respect to each other.

The view of propellers as rather rigid scaffolds suggests that the known WD structures could be used to

support de novo design studies. Two WD repeat proteins of particular interest appear to have no individual repeat specializations and have minimum length strand-connecting loops and turns (βTrcp [see references in Neer et al., 1994], and a new ten repeat protein from influenza A virus [GenBank accession number L28125]). These may provide wonderfully fixed platforms onto which one can attach de novo designed peptides with predicted catalytic functions.

There is at least one major mystery resulting from these new beautifully symmetric Gβ structures: clearly, one does not need a WD repeat sequence to form a propeller, nor do propellers encode any obvious common function. This is true even though one can superimpose the α carbons of the a, b, and c strands from all seven Gβ WD repeats onto two non-WD-containing propeller structures (porcine collagenase and methylamine dehydrogenase) within 0.9–2.2 Å root mean square deviation. What does the WD repeat provide to the β propeller structure that is so useful that it has been duplicated and adapted to so many other distinctive functions? The answer to the question is sure to shed new light on the function of a large number of important proteins.

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